August 02, 2004

EXTRA! EXTRA! READ ALL ABOUT IT!

TOP STORIES:

** "**NEW!!!**" denotes papers that are new to the manuscript update or have switched categories (ex. Submitted to In Press)

ABSTRACTS FOR UPCOMING MEETINGS:

-None at this time…stay tuned…abstracts for HAB XI coming soon

SUBMITTED:

** **NEW!!!** **UPTAKE AND ELIMINATION KINETICS OF BREVETOXIN IN BLOOD OF STRIPED MULLET (*Mugil cephalis*) AFTER AQUEOUS EXPOSURE TO *Karenia brevis*** (CL# 1141, Environmental Health Perspectives)… Ricky Woofter, Kirsten Brendtro , Christopher O. Miles and John S. Ramsdell

ABSTRACT

Striped mullet were used to characterize the kinetics brevetoxin uptake and elimination following aqueous exposure to *Karenia brevis*. Mullet were first exposed to sublethal densities of *K. brevis* (~250 000 cells/liter) for 4, 8, 12,

and 24 hr. At each time point a blood sample was applied to blood collection cards and then extracted and analyzed for brevetoxin using radioimmunoassay. Blood brevetoxin levels were elevated all four time points during exposure. Mullet were then exposed to varying densities of *K. brevis* culture and toxin measured in the water of the aquarium and blood of the fish. Even at concentrations of *K. brevis* having low brevetoxin concentrations (0.5 ng/ml) brevetoxin was detected in the blood after 8 hrs of exposure. At higher concentrations, brevetoxin was detected as early as 1 hr and increased to peak levels at 12 hr Toxin levels reached an equilibrium after 24 hr in the continued presence of *K. brevis* and during this time the mullet maintained a blood:water coefficient of 2.2. Striped mullet were next exposed to *K. brevis* for 10 hr and when transferred to fresh seawater blood levels of brevetoxin remained elevated decreasing by only 50% after 116 hr The rate of elimination fit best to a two-phase exponential decay with t1/2 of 12 and 266 hr This study demonstrates an effective means to monitor blood brevetoxin levels in finfish and provides a foundation to characterize biologically relevant levels of brevetoxin in other species impacted by red tide events.

**** QUANTITATIVE CIGUATOXIN ANALYSIS IN FORTY GREAT BARRACUDA (*Sphyraena barracuda*) COLLECTED FROM THE FLORIDA KEYS, USA: COMPARISON OF TWO DETECTION METHODS** (CL# 1132, Environmental Health Perspectives) … Marie-Yasmine Bottein Dechraoui, Jessica Tiedeken, Robert W. Dickey, H. Ray Granade, Renuka Persad, and John S. Ramsdell

ABSTRACT

Forty great barracuda (*Sphyraena barracuda*) were caught between September 2001 and January 2002 in Marathon Key (Florida, USA) and analyzed for ciguatoxin activity. Two specific complementary methods were used for this purpose: the sodium channel site-5 receptor binding assay, and the ouabain veratridine-dependent cytotoxicity assay. A Caribbean ciguatoxin (C-CTX-1) was tested in both assays and compared to the dose-response of the brevetoxin congeners PbTx-1 or PbTx-3 to determine their relative potency and investigate the possibility of using brevetoxins as an internal standard. Sigmoidal calibration curves with Hill coefficients of 1 were obtained for both toxin classes in the two assays. Our results demonstrated that C-CTX-1 has an 8-fold higher affinity for the sodium channel than brevetoxins and a 440-fold higher potency in the cytotoxicity assay. Analysis of fish extracts calibrated against brevetoxin standard curves showed a linear relationship (r^2=0.77, slope 1.056 ± 0.2) between the two detection methods. Among the forty barracuda tested, 60% contained ciguatoxin levels that were above the detection limit of the cytotoxicity assay (0.039 ppb C-CTX-1 equivalents), and 30% were above 0.25 ppb, a level potentially harmful to humans. The most toxic fish contained 2.1 ppb C-CTX-1 equivalents. No correlation between barracuda size and toxin concentration was observed.

IN PRESS:

** NEW!!! ULTRASENSITIVE DETECTION OF BLOOD DOMOIC ACID BY COMPETITIVE ELISA FROM BLOOD COLLECTION CARDS (CL# 1149, Toxicon) …

Jennifer Maucher and John Ramsdell

ABSTRACT

Domoic acid (DA) is a rigid analog of the excitatory amino acid produced by the diatom genus *Pseudo-nitzschia*, and a neurotoxin in humans. During blooms, DA can contaminate shellfish, as well as other filter feeding organisms, and can be transferred by ingestion to higher trophic levels, including marine mammals and humans. In 1998, DA was responsible for the mass mortality of over 400 central California sea lions that ate contaminated fish. More recently, DA has been implicated in the strandings of several whales off the coasts of Maine and Nova Scotia. The prevalence of this algal toxin and its effects on protected species makes measurement of domoic acid in living animals a necessary biomonitoring tool for the near future. Blood is an ideal sample from which to measure DA in living animals, because it is in equilibrium with all tissues and thus provides an accurate measure of exposure. Blood collection cards, similar to those used in nationwide human prenatal screening programs, have already been used for the extraction and detection of brevetoxin in blood from laboratory animals and, more recently, marine mammals. However, a difficulty unique to measuring DA in blood is the rapid rate (> 99% in 2 hrs) at which it is cleared from blood. This makes detection of DA after exposure a challenging issue for biomonitoring purposes. To meet this challenge, a method of detection with extremely high sensitivity and specificity was sought to make the use of blood collection cards and subsequent DA detection from them more feasible. Using a direct competitive ELISA (cELISA), we analyzed the blood of DA exposed mice after extraction from the blood collection cards. By using this highly sensitive assay in conjunction with a method for easy blood sample storage and extraction, this could be a very effective means of biomonitoring marine mammals in the field, as well as human populations.

** NEW!!! DIFFERENTIAL RESPONSES OF STRESS PROTEINS AND ANTIOXIDANT ENZYMES TO VARIOUS SOURCES OF CELLULAR STRESS IN THE FLORIDA RED TIDE DINOFLAGELLATE, Karenia brevis …(CL# 1137, Journal of Phycology) …

Jeanine Miller and Fran VanDolah

ABSTRACT

This study identifies stress proteins and antioxidant enzymes that may play a role in the survival strategies of the Florida red tide dinoflagellate, *Karenia brevis*. Heat shock protein 60 (Hsp 60), mitochondrial small heat shock protein (mitosHsp), chloroplastic small heat shock protein (chlsHsp), Mn superoxide dismutase (SOD), and Fe SOD were first identified by western blotting. The induction of these proteins in laboratory cultures in response to elevated temperatures, hydrogen peroxide, lead, or elevated light intensities was next assessed. In parallel, F_V/F_M, a measurement of photosynthetic efficiency and common proxy of cellular stress, was determined. Hsp 60, Fe SOD, and Mn SOD were induced following exposure to elevated temperatures, hydrogen peroxide, or lead. MitosHsp responded only to heat, whereas chlsHsp responded only to H_2O_2-induced stress. The expression of stress proteins and antioxidant enzymes appears to be a more sensitive indicator of heat or chemically-induced stresses than F_V/F_M. However, F_V/F_M decreased significantly in response to elevated light intensities that did not induce the expression of stress proteins. These results identify for the first time several stress proteins and antioxidant enzymes responsive to cellular stress in *K. brevis* and provide evidence for differential sensitivity of cellular organelles to various sources of stress.

** NEW!!! PHARMACOLOGICAL EFFECT OF MARINE TOXINS, BREVETOXIN AND SAXITOXIN, ON MURINE FRONTAL CORTEX NEURONAL NETWORKS (CL# 1134, Toxicon)…

Nadezhda V. Kulagina, Thomas J. O'Shaughnessy, Wu Ma, John S. Ramsdell and Joseph J. Pancrazio

ABSTRACT

Brevetoxins and saxitoxin, which are produced by marine dinoflagellates, are very potent neurotoxins targeting separate sites of α unit of voltage sensitive sodium channels. An attractive approach for marine toxin detection relies on pharmacological modulation of voltage-dependent sodium channels expressed in cells or tissues. While these function-based cellular assays exhibit sensitivity, they are typically slow and have limited potential use for field applications. Cultured neuronal networks grown on substrate integrated microelectrode arrays (MEAs) have emerged as a robust and sensitive approach for environmental threat detection. The present work describes the rapid effects of brevetoxin (PbTx-2) and saxitoxin on embryonic murine frontal cortex neuronal networks on MEAs. Network recording parameters such as mean spike rate, burst rate, burst duration, number of spikes per burst and spikes amplitude were analyzed before and after exposure to the toxins. Saxitoxin produced fast and reversible inhibition of all electrophysiological parameters with IC_{50} ranged between 1.2 and 2.2 nM. Although brevetoxinalso caused inhibition of most of the network electrophysiological parameters, it produced an increase in burst duration at low concentrations (EC_{50} = 15 ± 2 nM, n=4) followed by inhibition at higher ones (IC_{50} = 63 ± 4 nM, n=4). Exposure of frontal cortex networks to brevetoxin and saxitoxin also caused differential effects on spike amplitude. This work demonstrates that cultured neuronal networks not only could be used for pharmacological characterization of marine toxins but they also provide tool with unique properties for their detection.

**** DEVELOPMENT AND APPLICATION OF LSU rRNA PROBES FOR *Karenia brevis* IN THE GULF OF MEXICO, USA** (CL# 1109, Harmful Algae) … Tina Mikulski, Steve Morton and Greg Doucette

ABSTRACT

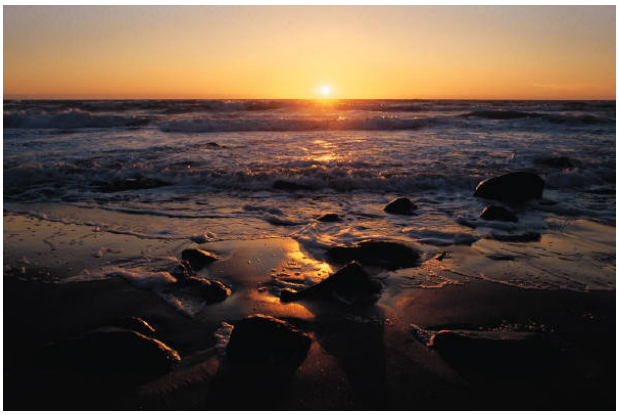
The brevetoxin producing dinoflagellate, *Karenia brevis*, is the target of several monitoring and research programs in the Gulf of Mexico where it forms extensive and frequently long-lived annual blooms that can cause severe economic losses. Rapid, reliable methods for the detection and enumeration of *K. brevis* cells, as well as their discrimination from morphologically similar species, are valuable tools for managers and scientists alike. Our aim was to produce a species-specific molecular probe that would serve as a tool to facilitate the efficient and reliable detection of *K. brevis* in the Gulf of Mexico. We sequenced a fragment of the large-subunit ribosomal RNA gene (LSU rDNA) from five *Karenia brevis* cultures isolated from the Texas Gulf coast, the Florida Gulf coast, and the Atlantic coast of Florida, and detected no differences among these isolates. A consensus sequence was thus compiled and compared to a previously published sequence from *K. mikimotoi*, the closest known phylogenetic relative to *K. brevis,* for the purpose of identifying unique *K. brevis* signature sequences. Fluorescently-labeled (FITC) oligonucleotide probes targeting these regions of the *K. brevis* LSU rRNA were designed to include at least two base pair differences, as compared to *K. mikimotoi*. Among seven probes designed, one uniquely identified all *K. brevis* isolates to the exclusion of all other species tested (Kbprobe-7), including a Gulf of Mexico *K. mikimotoi* isolate (Sarasota, FL) and several additional *Gymnodinium* species, as well as other dinoflagellate, diatom, and raphidophyte taxa. Importantly, *K. brevis* cells in samples taken during a 2001 bloom, fixed with a mixture of modified saline ethanol and 10% formalin, and stored at 4°C for seven months were successfully labeled with Kbprobe-7. In addition, preliminary analysis of labeled cells by flow cytometry revealed that *K. brevis* could be distinguished from *K. mikimotoi* in solution, suggesting other potential applications of this probe.

**** EXPRESSION OF A α,β,γ TUBULIN, THE MINIMAL SET OF TUBULINS REQUIRED TO DEFINE MICROTUBULE FUNCTION IN EUKARYOTIC CELLS, IN THE UNICELLULAR DINOFLAGELLATE, *Karenia brevis*.** (CL# 1045, Phycologia) … Michèle Barbier et al. (Jeanine Miller, Steve Morton and Fran VanDolah)

ABSTRACT

Tubulin is a highly conserved family of proteins that are a major component of the microtubule cytoskeleton of eukaryotic cells. Here we report the presence of the three essential members of this family, α-, β- and γ-tubulin, in the unicellular dinoflagellate *Karenia brevis* by western blotting and immunolocalization. The cortical cytoskeleton and the intracytoplasmic structures are detailed by immunocytofluorescence techniques using antibodies to each tubulin on whole-permeabilized cells from laboratory cultures or field samples. The cortical microtubules could be

visualized with anti-α- and anti-β-tubulin labeling revealing a morphology typical of dinoflagellates, while γ-tubulin was detected near the nucleus, probably associated with the archoplasmic sphere. The mitotic spindle, which arises from this region is described during different stages of mitosis. The cortical cytoskeleton does not depolymerize during mitosis, a feature that appears to be unique to dinoflagellates. For the first time, a detailed description of the cytoskeleton and the mitotic process is presented in the dinoflagellate *K. brevis*.



PUBLISHED:

**** NEW!!! CHARACTERIZATION OF THE DEVELOPMENTAL TOXICITY OF CARIBBEAN CIGUATOXINS IN FINFISH EMBRYOS** (CL# 1124, Toxicon 44(1): 56-66) … Jamie R. Colman, Marie-Yasmine Bottein Dechraoui, Robert W. Dickey, and John S. Ramsdell

ABSTRACT

Since oviparous fishes mobilize fat stores to produce eggs, we investigated the potential for deposition of gonadal ciguatoxins to the oil laden yolk sacs which nourish developing embryos, and characterized the effects of these toxins on finfish development. Results showed that ciguatoxins are more concentrated in the egg mass (0.18 ng/g) of a toxic fish than in the muscle (<0.04 ng/g). We used a microinjection technique in a Japanese medaka (*Oryzias latipes*) developmental fish model to mimic the maternal route of toxin exposure to finfish embryos. We describe the developmental effects of two preparations isolated from Caribbean great barracuda (*Sphyraena barracuda*): a highly purified toxin (C-CTX-1), and ciguatoxins extracted from the flesh of a toxic fish. C-CTX-1 induced a significant decrease in heart rate after four days, which did not persist with further development. Crude extracts from ciguatoxic fish flesh induced hyperkinetic twitching and severe spinal deformities. These effects were observed in embryos receiving as little as 5 pg/egg, and were consistently found in embryos receiving doses exceeding 10 pg/egg. The occurrence of twitching and spinal deformities increased in both frequency and severity with dose. Larvae suffering from spinal abnormalities were unable to orient themselves, and could not feed, resulting in mortality. The greater distribution of toxin to eggs as compared to flesh suggests that fish with low to moderate (0.5 ppb) flesh toxin levels would maternally transfer detrimental amounts of ciguatoxins to their offspring.

**** THE TYPE B BREVETOXIN (PbTx-3) ADVERSELY AFFECTS DEVELOPMENT, CARDIOVASCULAR FUNCTION AND SURVIVAL IN MEDAKA (*Oryzias latipes*) EMBRYOS.** (CL# 1099, Environmental Health Perspectives 111(16): 1920-1925) … Jamie Colman and John Ramsdell

ABSTRACT

Brevetoxins are produced by the Florida red tide dinoflagellate, *Karenia brevis*. The toxins are lipophilic polyether toxins, which elicit a myriad of effects depending upon the route of exposure. Brevetoxins are therefore broadly toxic to marine and estuarine animals. By mimicking the maternal route of exposure to the oocytes in finfish, we have characterized the adverse effects of the type 1 brevetoxin, brevetoxin (PbTx-3), on embryonic fish development and survival. The Japanese rice fish, Medaka (*Oryzias latipes*) was used as the experimental model in which individual eggs were exposed via microinjection to known concentrations of PbTx-3 dissolved in natural fish oil. Embryos injected with doses exceeding 1ng/egg displayed tachycardia, hyperkinetic twitches in the form of sustained convulsions, clumping of the erythrocytes, and decreased hatching success. Furthermore, fish dosed with toxin were often unable to hatch in the classic tail first fashion and emerged head first, which resulted in partial hatches and death. An LD_{50} was determined wherein a dose of 4.0ng was sufficient to kill 50% of the fish, resulting in death. The results of this study complement previous studies of the developmental toxicity of the type 2 brevetoxin, brevetoxin-1 (PbTx-1). Furthermore, they provide information on the most commonly occurring brevetoxin and will illustrate *in vivo* the differing toxicities between the type 1 and type 2 brevetoxins.

** PERMANENT EXPRESSION OF A CYCLIN B HOMOLOGUE IN THE CELL CYCLE OF THE DINOFLAGELLATE, *Karenia brevis* …

(CL# 1037, Journal of Eukaryotic Microbiology 50(2): 123-31) Michèle Barbier, Tod Leighfield, Soyer-Gobillard, M.O. and Fran VanDolah

ABSTRACT

The eukaryotic cell cycle is driven by a set of cyclin dependent kinases associated with their regulatory partners the cyclins, which confer activity, substrate specificity and proper localization of the kinase activity. We describe the cell cycle of *Karenia brevis* and provide evidence for the presence of a cyclin B homologue in this primitive eukaryotic dinoflagellate. This cyclin B homologue has an unusual behavior, since its expression is permanent and its localization is cytoplasmic throughout the cell cycle. This behavior is similar to a cyclin B homologue, p56, previously described in a different species of dinoflagellate. However, in *K. brevis*, the cyclin B homologue is also present in the nucleus, specifically bound to the nucleolus during interphase. There is no evidence for the translocation to the nucleus during mitosis. Here we discuss the unique behavior of the cyclin B homologue in dinoflagellates, its relationship to the unusual characteristics of dinomitosis, and its potential implications regarding the evolution of cell cycle regulation among eukaryotes.

** MEASUREMENT OF BREVETOXIN LEVELS BY RADIOIMMUNOASSAY OF BLOOD COLLECTION CARDS AFTER ACUTE, LONG-TERM AND LOW DOSE EXPOSURE IN MICE.

(CL# 1080, Environmental Health Perspectives 111(13): 1595-1600) …
Ricky Woofter, M-Yasmine Bottein Dechraoui, Ian Garthwaite, Neil R. Towers, Christopher J. Gordon , José Córdova and John S. Ramsdell

ABSTRACT

A radioimmunoassay (RIA) has been developed using a sheep anti-brevetoxin to evaluate detection of brevetoxin on blood collection cards from mice treated with the brevetoxin congener (PbTx-3). The RIA was designed in similar format to receptor assay to facilitate comparison with previous work with blood collection cards. The RIA uses a 1/4000 dilution of sheep antiserum, 0.4 nM [^{3}H]-PbTx-3, and goat antisheep IgG-cellulose with separation on glass fiber filters. The receptor binding assay (RBA), using rat brain membrane, has an affinity for PbTx-3 (EC_{50}= 4.3 ± 1.5 nM, n=7) and recognizes type 1 and type 2 brevetoxins, as well as ciguatoxin. Whereas the RIA, using a PbTx-2 specific antibody, has an affinity for PbTx-3 (EC_{50}= 1.2 ± 0.2 nM, n=10) and recognizes both type 1 and type 2 brevetoxins, but not ciguatoxin. Comparison of the different brevetoxin subtypes affinity using RIA and RBA yields a rank order of potency where PbTx 6 > 3 = 2 = 9 > 1. Thus, the two assays provide comparable values for the commonly occurring PbTx-2 and 3 as well as PbTx-9, while showing differences for PbTx-6 and PbTx-1. We next compared the two assays by measuring brevetoxin in the blood of mice exposed to a sublethal dose, 180 µg/kg of PbTx-3 for 0.5, 1, 2, 4, and 24 hr. The blood from each mouse was preserved on blood collection cards. Each 0.1 ml blood spot was extracted in 2 ml methanol. This extract was then tested by both assays. The RBA reported the blood brevetoxin activity (at 2 hr brevetoxin activity was detected in 3 of 4 mice), while the RIA gave blood

brevetoxin levels (at 2 hr: 25.75, 28.27, 39.26, 28.51 nM PbTx-3). Taken together these results show the value of tier-based testing for brevetoxin: antibody methods provide a good screening method that may detect metabolites; receptor-based methods provide a good toxicity measurement and LC-MS/MS provides absolute confirmation of toxin congeners.

**** RE-EVALUATION OF PARALYTIC SHELLFISH TOXIN PRODUCTION BY BACTERIA ASSOCIATED WITH DINOFLAGELLATES OF THE PORTUGUESE COAST**. … (CL# 1085, Applied and Environmental Microbiology 69: 5693-5698) Martins, C.A., Alvito, P., Tavares, M.J., Pereira, P., Doucette, G.J. and S. Franca

ABSTRACT

Paralytic Shellfish Toxins (PSTs) are a suite of potent neurotoxins whose production is associated with certain dinoflagellate and cyanobacterial species. The autonomous production of PSTs by some bacterial strains, namely those associated with PST producing dinoflagellates, remains controversial. In addition to reports on their PST production, there is some evidence to suggest that certain compounds in some bacterial isolates were incorrectly identified as PSTs by HPLC analysis. In the current study, PST production by two bacterial strains, *Pseudomonas stutzeri* and *Pseudomonas diminuta*, isolated from *Alexandrium lusitanicum* and *Gymnodinium catenatum*, respectively, was evaluated using a mouse neuroblastoma (MNB) assay and the results compared to HPLC analyses of the same samples. Since we have previously assessed the presence of PSTs in these bacterial isolates by HPLC, results of the present study are also discussed in relation to our earlier findings. Toxicity studies were performed under optimal conditions for toxin production and detection, as described in the published literature. PSTs were not detected by HPLC analysis in either supernatants or bacterial cell extracts. Analysis by MNB assay was negative for supernatants but initially positive for crude extracts. Nonetheless, this positive assay response was eliminated following C18 sep-pak clean-up of the extracts, indicative of a matrix effect on the assay and thus the absence of PSTs in these samples. We conclude that neither our MNB nor HPLC data are consistent with autonomous bacterial PST production under the study conditions.

**** *IN VITRO* ASSAYS FOR PHYCOTOXINS…** (CL# 1038, In: Hallegraeff, G.M., Anderson, D.M. & Cembella, A.D. (eds.), *Manual on Harmful Marine Microalgae*. Second Edition. Monographs on Oceanographic Methodology, 11. IOC-UNESCO, Paris. pp. 297-345.) **…**A.D. Cembella, Greg Doucette and Ian Garthwaite

ABSTRACT

Not available in electronic form at this time (see Greg)